

Effects of enzyme induction on metabolism of prednisolone

Clinical and laboratory study

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Brooks, P. M., Buchanan, W. W., Grove, M., and Downie, W. W. (1976). *Annals of the Rheumatic Diseases*, 35, 339-343. Effects of enzyme induction on metabolism of prednisolone. Clinical and laboratory study. The addition of phenobarbitone in therapeutic dosage to the drug regimen of prednisolone-treated subjects with rheumatoid arthritis produced measurable deterioration in the clinical status of the patients associated with a more rapid clearance of prednisolone from plasma. It is considered that phenobarbitone induced the hepatic metabolism of prednisolone, effectively reducing the steady state plasma level and resulting in clinical relapse. A slight but significant improvement in the adrenocortical response to tetracosactrin (Synacthen) was noted after phenobarbitone therapy.

The list of drugs capable of inducing increased synthesis of hepatic microsomal enzymes is growing rapidly and includes barbiturates, diphenylhydantoin, glutethimide, meprobamate, phenylbutazone, tolbutamide, and ethanol (Stevenson, 1973). Most attention has been focused on the effects of enzyme induction on concomitant drug therapy with reduction in therapeutic efficacy, e.g. warfarin anticoagulation (MacDonald and others, 1969). However, enhanced metabolism of endogenous substrates such as bilirubin (Kreek and Sleisinger, 1968) may also occur with important therapeutic effects. The rate of metabolism of endogenous cortisol is increased by diphenylhydantoin. Reduction in the plasma half-life of ^{14}C -cortisol has been noted, with a compensatory increase in the cortisol secretion rate (Werk, MacGee, and Sholiton, 1964; Choi and others, 1971). It has been suggested that phenytoin may be used as a nonsurgical method of treating Cushing's syndrome (Conney, 1967). An interesting observation after induction of cortisol metabolism is the excretion in urine of increased amounts of 6β -hydroxycortisol (Werk and others, 1964). This has become a recognized endogenous marker of hepatic microsomal enzyme induction (McEwen and Stevenson, 1971).

Prednisolone has been available for clinical use since 1955 (Herzog and others, 1955b). Its effectiveness as an antirheumatic agent was quickly established (Herzog and others, 1955a; Bunim and others, 1955; Hart, Clark, and Golding, 1955). Little is known about its metabolism. A considerable degree of hepatic conversion occurs (Vermeulen, 1959), and several studies using nonspecific techniques have suggested that the plasma half-life of prednisolone is longer than that of cortisol. Values for prednisolone range from 192 minutes (Ely, Done, and Kelly, 1956) to 200-220 minutes (Sandberg and Slaunwhite, 1957; Nugent, Eik-Nes, and Tyler, 1959) compared with cortisol with half-life times of between 80 and 113 minutes (Sandberg and Slaunwhite, 1957; Nugent and others, 1959). The heightened potency of the synthetic steroid has been attributed at least in part to its slower degradation.

Multiple drug use is one of the problems of current therapeutics, and is a major factor in the high prevalence of adverse drug reactions (Wade, 1970). The introduction of an enzyme-inducing agent to an established drug regimen might theoretically enhance the clearance of other drugs metabolized in the liver and lead to a deterioration in the patient's clinical status.

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Phenobarbitone is still widely prescribed in general practice for night sedation (Dunlop, 1971) and in a recent study on inpatient prescribing at a rheumatic diseases centre, 5 of 153 patients were being prescribed barbiturates on admission to hospital. Because of the known stimulation of native corticosteroid metabolism by enzyme-inducing drugs (Choi and others, 1971) it was decided to observe the clinical and metabolic effects of the administration of phenobarbitone 60 mg twice daily to prednisolone-treated patients with rheumatoid arthritis.

Materials and methods

Nine patients with classical or definite rheumatoid arthritis according to the criteria of the American Rheumatism Association (Ropes and others, 1959) were studied before and after the addition of phenobarbitone 60 mg twice daily to their therapeutic regimen. The mean age of the patients was 50.5 years (range 42–65 years) and the mean duration of disease activity was 16.55 years (range 11–28 years). All had been on prednisolone therapy for at least 6 months with no change in dosage and the mean daily dosage of prednisolone was 10.8 mg (± 2.61 mg SD) with a range of 8 mg–15 mg.

Patients were asked to record the duration of morning stiffness and their daily pain score on a pain self-assessment chart (Lee and others, 1973) for 3 days before the study began and for the 14 days of phenobarbitone therapy. Clinical assessment of disease activity, using the articular index of joint tenderness (Ritchie and others, 1968) and the grip strength of the right and left hands (Lee and others, 1974) was performed before and after phenobarbitone treatment. Clinical indices were determined by a single observer at the same time of day on each occasion. A mean was taken of the duration of morning stiffness and the average pain score on the 3 days before starting phenobarbitone and the final 3 days of phenobarbitone therapy.

Before the introduction of phenobarbitone therapy and at the end of the 2-week test period, the prednisolone half-life was estimated. 5 μ Ci tritiated prednisolone, labelled in the C₁ and C₂ positions on the A ring (Radiochemical Centre, Amersham), was administered intravenously to the patient. Serial venous blood sampling at 30-minute intervals up to 180 minutes was performed. The steroid from 2 ml plasma from each sample was extracted with 15 ml methylene dichloride and a 10 ml

aliquot taken to dryness in B14 tubes in a cool water bath. The residue was dissolved in 3 \times 0.2 ml aliquots of a 10% ethyl alcohol and toluene solvent mixture. Chromatography was performed using columns containing 1G Sephadex LH-20-100. Plasma extract was added to the column and eluted with 20 ml 10% ethyl alcohol and toluene. Selective fraction collection was performed. Fraction 4–8 ml contains prednisone, and 9–18 ml contains the prednisolone. The fractions were collected in glass vials and evaporated to dryness. 10 ml liquid scintillator was added to each vial and counting (10-minute counts) carried out on a Nuclear Enterprises liquid scintillation counter. The half-life of prednisolone was calculated using the counts per minute per 100 ml plasma from each of the prednisolone-containing fractions of each blood sample, using the method of 'least squares'.

Selection of the appropriate fractions was previously calculated by separation of a mixture of 0.2 ml tritiated prednisolone (0.5 μ Ci) and 0.2 ml 'cold' prednisone (1 mg/ml) dissolved in 10% ethyl alcohol and toluene and eluted with 20 ml solvent as previously described. One ml fractions were collected and further divided into 2 \times 0.5 ml aliquots. One 0.5 ml fraction from each sample was processed for liquid scintillation counting, and the parallel sample subjected to the blue tetrazolium reaction (Nowaczynski, Goldner, and Genest, 1955). Radioactivity was first detected in sample 9, increasing counts being noted up to sample 15, and eventually disappearing at sample 19. After reaction with blue tetrazolium, each sample was read on the SP500 spectrophotometer at 510 nm. Increase in optical density was first noted in sample 4, increasing to a maximum in sample 7, and was virtually undetectable in sample 9.

The addition of phenobarbitone in varying concentrations did not significantly alter the extraction of steroid from plasma during *in vitro* studies. Phenobarbitone concentrations ranging from 0.2 mg/100 ml (0.86 μ mol/l) (the upper limit of the anticonvulsant therapeutic effect) were used. None of the patients in our study exceeded a steady state level of phenobarbitone greater than 1.8 mg/100 ml (77.6 μ mol/l). No specific studies of binding of prednisolone to plasma proteins were carried out. It is now generally accepted that changes in binding are irrelevant unless a drug is bound to more than 95%. Estimates of plasma binding of prednisolone suggest a figure not exceeding 90%.

After the venous blood sampling at 180 per minute, 250 μ g tetracosactrin (Synacthen, Ciba) was injected intravenously and a further venous sample removed after 30 minutes (210 minutes after the original tritiated pred-

Table I *Results of clinical assessment*

	Before phenobarbitone (mean \pm SEM)	After phenobarbitone (mean \pm SEM)	P
Articular index	21.7 \pm 4.04	28.5 \pm 3.99	<0.025
Pain score	1.29 \pm 0.15	2.66 \pm 0.36	<0.0025
Morning stiffness (h)	0.86 \pm 0.16	1.87 \pm 0.24	<0.005
Grip strength (mmHg)			
Right hand	110 \pm 25	101 \pm 27	NS
Left hand	118 \pm 26	106 \pm 29	NS

NS = not significant.

Table II Results of laboratory assessment

	Before phenobarbitone (mean \pm SEM)	After phenobarbitone (mean \pm SEM)	P
Prednisolone half-life (min)	132.0 \pm 15.2	99.3 \pm 10.7	<0.0025
Plasma 11-OHCS level (μ g/100 ml baseline)	10.6 \pm 0.89	11.6 \pm 1.1	NS
Plasma 11-OHCS level (μ g/100 ml) (30 min after tetracosactrin)	11.4 \pm 1.13	13.6 \pm 2.0	<0.02

11-OHCS = 11-hydroxycorticosteroids.

nisolone injection). Estimation of the plasma 11-hydroxycorticosteroids (11-OHCS), by the method of Mattingly (1962), was performed on the 180- and 210-minute plasma samples.

Analysis of all parameters of clinical status, prednisolone half-lives, and plasma 11-OHCS levels before and after phenobarbitone administration was carried out using Student's 't' test for paired values.

Results

Table I shows the results of the clinical assessment. Deterioration in joint tenderness was evidenced by a significant rise in the articular index from a mean of 21.7 before phenobarbitone to 28.5 after phenobarbitone. Further evidence of clinical deterioration was shown by an increase in the pain score from a control value of 1.29 to a level of 2.66 at the end of the trial period. The duration of morning stiffness increased from a mean value before phenobarbitone of 0.86 hours to a value after phenobarbitone of 1.87 hours. A fall in grip strength was noted but failed to reach statistical significance.

The results of the laboratory analyses are shown in Table II. A 25% reduction in the half-life of prednisolone was found, falling from a mean control value of 132 minutes to a value after phenobarbitone of 99.3 minutes. No alteration in the volume of distribution of the tritiated prednisolone was noted. The half-life shortened in 8 of the 9 subjects studied and remained unchanged in the remaining patient.

No significant difference was noted in the mean baseline plasma 11-OHCS levels before or after phenobarbitone (control 10.6 μ g/100 ml; after phenobarbitone 11.6 μ g/100 ml). Before administration of phenobarbitone the mean plasma 11-OHCS level 30 minutes after tetracosactrin was 11.4 μ g/100 ml. At the conclusion of the trial, the mean 30-minute post-tetracosactrin 11-OHCS value was 13.6 μ g/100 ml. This was significantly higher than the comparative control level. In one patient there was no response to tetracosactrin at the control stage of the experiment, but after 2 weeks of phenobarbitone therapy the plasma 11-OHCS response to tetracosactrin rose into the accepted normal range (Wood and others, 1965).

At the end of the trial period the mean phenobarbitone level in plasma for the group was 1.43 mg/100 ml (\pm 0.30 SEM) (61.7 μ mol/l \pm 12.9).

Discussion

An important finding is that the plasma half-life of prednisolone, measured by the technique described, is considerably shorter than previously documented. The mean value in the current study is just over 2 hours compared with values between 3 and 4 hours reported previously (Ely and others, 1956; Sandberg and Slaunwhite, 1957; Nugent and others, 1959). One possible explanation may lie in the more specific methodology employed in the present study, particularly the chromatographic separation of prednisolone from its metabolites. The half-life line is constructed from the disappearance rate of radioactivity associated with prednisolone, and excluding counts associated with the major metabolite prednisone. Unfortunately, due to lack of information regarding other circulating metabolites of prednisolone, we could not establish the purity of the prednisolone fraction. However, separate counting of the fractions of the prednisolone aliquot in 4 subjects indicated a smooth rise to the count peak and a similar fall suggesting that the bulk of radioactivity was associated with a single compound. Further work needs to be carried out on separation techniques but the method currently used does seem to demonstrate changes in half-times of radioactive prednisolone.

Notwithstanding possible imperfections in the counting technique, another explanation for the discrepancy in the half-life values may lie in the differences in steroid dosage employed. Early studies were carried out on subjects receiving large doses of prednisolone intravenously or taking massive doses, e.g. 80 mg per day by mouth. It is known that metabolism of prednisolone follows first order kinetics, and it is possible that the large amounts of prednisolone used previously saturated the metabolic capacity of the hepatic microsomes, leading to apparently long half-lives. It is interesting

that the control values for prednisolone half-lives found in the present study in subjects receiving low dose oral therapy are not significantly different from values found in age- and sex-matched volunteers who received only a tracer dose of prednisolone intravenously (W. W. Downie, unpublished, 1975). This supports the suggestion that at a critical plasma level saturation kinetics may apply to prednisolone. Until a reliable method is available for measuring steady state plasma levels of prednisolone this question will remain unanswered.

The results indicate that shortening of the plasma half-life of prednisolone is produced by administration of phenobarbitone in therapeutic doses. No change in the volume of distribution was noted, suggesting that the reduction in half-life is a feature of enhanced metabolism rather than a distribution phenomenon. The level of serum albumin may be important in determining the relative percentages of 'free' and bound prednisolone (Lewis and others, 1971). Low albumin levels are associated with a higher percentage of 'free' and hence biologically active steroid, resulting in enhanced toxicity.

No estimations of protein binding nor of specific steroid binding globulin were carried out in the present study but results of similar experiments on cortisol metabolism have indicated that neither percentage binding nor binding protein levels were altered by enzyme-inducing drugs such as diphenylhydantoin (Choi and others, 1971).

The quantifiable clinical deterioration noted during phenobarbitone administration is unlikely to represent the natural fluctuations of activity of the arthritis since the deterioration was noted in 8 of the 9 patients studied and did not occur in the single patient whose half-life remained unchanged. The

likeliest explanation is that increases in the rate of prednisolone clearance will reduce circulating and tissue levels of the drug, producing in effect a reduction in dosage with subsequent deterioration in clinical status. A similar deterioration in ventilatory function in dexamethasone-treated asthmatic subjects has been shown after introduction of diphenylhydantoin (Brooks and others, 1972). For this reason it is suggested that a change in disease activity after introduction of additional therapy should be considered to have an iatrogenic component related to changes in established drug metabolism rather than be dismissed casually as a naturally occurring relapse or remission.

The changes in endogenous adrenocortical function are not dramatic but the overall improvement in the plasma 11-OHCS response to tetracosactrin after phenobarbitone, and in particular the dramatic improvement shown by one subject, suggests that an enzyme-inducing agent may be useful in reversing the most important side effect of corticosteroid drug therapy, namely suppression of the activity of the cerebro-hypothalamo-pituitary-adrenal axis (Nuki and Downie, 1971). Failure to produce a more dramatic degree of improvement in adrenocortical function in our subjects may be related to the duration of therapy, the relatively high dosage schedules of prednisolone, or the low dosage of phenobarbitone employed.

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